EXPERIMENTS ON CUTTING OFF PARTS OF THE COTYLEDONS OF PEA AND NASTURTIUM SEEDS.

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THE experiments to be described were undertaken as bearing upon the general problem of the relation of food supply to growth. They were carried on under the guidance of Professor T. H. Morgan, to whom I am much indebted for aid and suggestions. The variations in food supply were produced in the pea and nasturtium, both dicotyledonous plants, by cutting off part of the cotyledons, thereby reducing the amount of food stored up by the parent plant for the use of the seedling. The questions that arise relate to the effect upon the size of the seedling, upon the differentiation of its organs, and upon the number and size of the component cells, caused by thus reducing the food supply. These questions may be answered from the results of the experiments.

As already stated, the pea and the nasturtium were the plants selected. Before deciding on them, however, the morning-glory, sweet-pea, radish, common bean, buckwheat, mustard, cucumber, and pumpkin were tested as to their suitability, by planting specimens of each with portions of their cotyledons cut off. It was found that the peas and nasturtiums, possessing large cotyledons, were more easily manipulated, and that their seedlings were hardier than those of the other plants. Their seedlings, moreover, grew rapidly, so that differences in the relative size of the plants were early noticeable and were well marked. Under favorable conditions, however, good results might be obtained from some of the other species, and it would be interesting to see to what extent they corroborate those from the two plants here discussed.

The seeds tested for availability, and subsequently all the pea and nasturtium seeds, were treated as follows : After they

had been soaked in water for from twelve to twenty-four hours to soften them, their seed-coats were removed and, from some of the seeds, parts of the cotyledons were cut off with a sharp scalpel, while the others were left in their normal condition save for the removal of their seed-coats. The normal seeds underwent the soaking in water and removal of the seed-coats to make their condition like that of the others except in the one point of food supply. When thus prepared, all these seeds were planted on sawdust, which was kept wet during their growth. In the case of the pea and nasturtium, as soon as the plants from these seeds were well started a second lot of normal seeds, treated in the same way as the normal seeds described above, was planted. In a short time, usually about two weeks, the plants of the second lot were found to be about the same size as those of the first lot that had come from the reduced seeds. Sections of the stems were then cut freehand, and camera drawings made.

The amount of cotyledon cut off from the seeds varied very considerably, and was not in all cases quantitatively determined. The variation may be seen from Table I, where are

TABLE I.

Part by Weight left, expressed in Percentages.	No. of Peas.	No. of Nasturtiums.	Part by Weight left, expressed in Percentages.	No. of Peas.	No. of Nasturtiums.
10–15	—	2	31–35	14	5
16–20	10	9	36–40	5	1
21–25	8	9	41–45	3	1
26–30	16	8	46–50	1	—

represented the percentages by weight of the part left in the case of 57 peas and 35 nasturtiums. The seeds were weighed after the removal of the seed-coats and again after the removal of part of the cotyledons, and the percentages express the ratio between the two weights. The percentages in the case of the peas vary from 16 to 48, with more than half the individuals between 26% and 35%; those in the case

of the nasturtiums vary from 10 to 41, with more than half the individuals between 16% and 25%, and nearly three-quarters between 16% and 30%. The chances, then, that from 65% to 74% of the bulk of any pea seed has been removed, are even; and the chances are three to one that from 70% to 84% of any nasturtium seed has been removed.

The variation in the amount of cotyledon removed appeared to influence the rate of growth of the seedling, but the number of plants of which a quantitative record of the development was kept was too small to justify an attempt to lay down a rule concerning the extent of this influence. The seedlings will therefore be regarded as belonging to only two classes, the normal and the dwarf, the latter composed of plants growing from seeds that have been reduced by removing part of their cotyledons. Plants of the two classes sprouted at about the same time, and for a short time the differences between them were not striking. As soon, however, as leaves began to develop, the normal seedlings shot ahead, surpassing the dwarf seedlings not only in size, but also in the number and size of their leaves. A comparison of two groups of pea plants, as given in Table II, from readings taken at three different times, shows the relative rate of development. The figures represent the height of the plants in millimeters and their number of leaves, while at the foot of each column is placed the average of the readings in that column. The readings from the dwarf seeds are arranged in the order of the fraction of the seed that is used for planting, and the readings from the normal seeds, in the order of the weight of the seeds, the smallest fraction and the smallest weight being at the top of their respective columns. The first period, two weeks after the seeds were planted, corresponds to an early stage of development; the second, five weeks old, is the stage just before the production of flowers by the normal plant; and the third period, nine weeks old, is the period of maturity, when the plants are bearing flowers and seeds. The letters *fl.* and the word *pod* in the column marked "leaves," mean that the plant against which they are placed has a flower or a seed-pod.

Table II.

	Two Weeks.				Five Weeks.				Nine Weeks.			
	Dwarf.		*Normal.*		*Dwarf.*		*Normal.*		*Dwarf.*		*Normal.*	
	mm.	Leaves.	mm.	Leaves.	mm.	Leaves.	mm.	Leaves.	mm.		mm.	
a	17	5	35	11	22	9	77	23	35	—	95	fl.
b	10	—	29	11	29	9	70	19	47	—	89	—
c	16	5	32	13	35	15	86	27	44	—	108	pod.
d	22	7	32	11	26	9	77	27	35	—	102	fl.
e	26	11	32	11	54	20	89	27	70	fl.	117	pod.
f	26	9	38	13	47	17	77	27	63	fl.	95	—
g	29	9	41	13	60	21	92	28 fl.	77	pod.	108	pod.
Av. . . .	22	7	34	12	39	14	81	25	53		102	

From Table II we may collect several facts as to the relations in size and development between dwarf and normal plants. In the first place, the ratio of height of dwarf to height of normal, expressed in percentages, varies as follows: At two weeks, 65%; at five weeks, 36%; at nine weeks, 52%. The ratios between the number of leaves of the dwarf and of the normal are 58% and 56% for the two and five weeks old plants, respectively. No readings were made of the number of leaves at the third stage, because some of the plants had lost leaves, and the oldest leaves of all the plants were beginning to wither, so that the number had not as much significance as in the earlier stages. Similar measurements of height and number of leaves made on another lot of peas showed similar variations in ratios, so we may consider the readings here given as typical. The advantage in size, then, which the normal seedlings show in the first stage becomes more marked as the plants grow older, while the differentiation, so far as expressed by the number of leaves, though markedly greater in the normal than in the dwarf plant, does not increase much faster in proportion in the former than in the latter. The time of the first appearance of flowers is, however, more significant of the differentiation of the plant than is the number of leaves, for the latter may depend on the size of the plant, as well as on its degree of development. The time of flowering was unmistakably earlier in the normal plants, for the first flower appeared upon them at five weeks, whereas the first flowers appeared on the dwarf plants at eight weeks. At this time three dwarf plants blossomed, while during the same period five of the normal plants had borne flowers, of which three had developed into pods. These facts suggest the conclusion that the normal plants not only are larger than are the dwarf plants but develop more quickly than do the latter.

It remains, therefore, to be seen whether the foregoing conclusions to which the macroscopic examination has led are supported by the examinations of sections under the microscope. An examination of this sort may be looked to to answer the following four questions:

A. What is the relative size of the cross-section of a normal and of a dwarf plant?

B. What is the relation between the number of cells in a normal cross-section and a dwarf one?

C. What is the relation between a normal and a dwarf plant, as regards the size of the cells?

D. How does the degree of differentiation of the normal compare with that of the dwarf plant?

Attempts were made to compare sections of the dwarf seedling under the microscope with sections of a normal seedling of the same size, as a check, as well as with the normal seedling of the same age, but the only specimens of check seedling available for comparison were so much larger than the dwarf that allowance must in every case be made for a discrepancy. The cross-sections studied were all cut free-hand from a level less than an inch from the ground in the growing plant.

The first question in the preceding paragraph relates to the relative size of the cross-section of the stem of the normal and dwarf plants. In the cross-sections represented by the figures the diameters of the peas have the relative values of 36 and 53, or the diameter of the dwarf pea is .68 as great as the diameter of the normal; the diameters of the nasturtiums have the relative values of 30 and 38, or the diameter of the dwarf is .79 as great as that of the normal. Cross-sections of the stem of the check plants have the values 46 and 33 for the diameters of the pea and nasturtium, respectively. The dwarf and normal plants were five weeks old and the check plant two and one-half weeks old. The plants selected for examination were typical ones, and the fact that the ratio between the diameters does not correspond to the average ratios between the heights given in Table II may be explained in two ways:

1. The ratio between the heights, as was seen, decreases with the increasing development of the plants, and the degree of development of the plants from which the cross-sections were taken was probably not the same as that of the plants measured in Table II.

2. The stem of small plants is always thicker in proportion to the size of the plant than that of large plants, so less difference in cross-section than in height is to be expected. Interpret this discrepancy as we may, the fact remains that the stem of a normal plant has a greater diameter than the stem of a plant sprung from a seed part of whose cotyledons has been cut off.

The ratio between the number of cells in a normal cross-section and a dwarf cross-section can be determined by counting the number in a definite sector of each. The results of counting the number of cells in a sector of 30° are: in the normal pea (Fig. 1), 410 cells; in the dwarf pea (Fig. 2), 311 cells; in the normal nasturtium (Fig. 3), 223 cells; in the dwarf nasturtium (Fig. 4), 208 cells; in the check pea, 404 cells; in the check nasturtium, 219 cells. The dwarf plant has, therefore, decidedly fewer cells than the normal; in the case of the pea .76 as many, and in the case of the nasturtium .93 as many. If the ratio between the number of cells was the same as the ratio between the diameters of the cross-sections, it would mean that the cells must be of the same size; since the former ratio is larger for both pea and nasturtium, it means that the cells of the normal are larger than those of the dwarf plant.

The conclusion as to the size of cells in dwarf and normal plants may be confirmed directly by counting the number of cells in a definite area. Proceeding to do this for Parenchyma cells, the following results were obtained: In the normal pea (Fig. 1), 22 cells; in the dwarf pea (Fig. 2), 43 cells; in the normal nasturtium (Fig. 3), 21 cells; in the dwarf nasturtium (Fig. 4), 32 cells; in the check pea, 50 cells; in the check nasturtium, 29 cells. The regions counted were all in the same part of the stem, and other counts were made that corroborate the figures here given. These figures confirm the conclusion reached in the preceding paragraph as to the size of the cells in normal and dwarf plants; but in the case of the check pea plant it is found that the cells are smaller than those of the dwarf plant.

Though the statistics from microscopic examination that

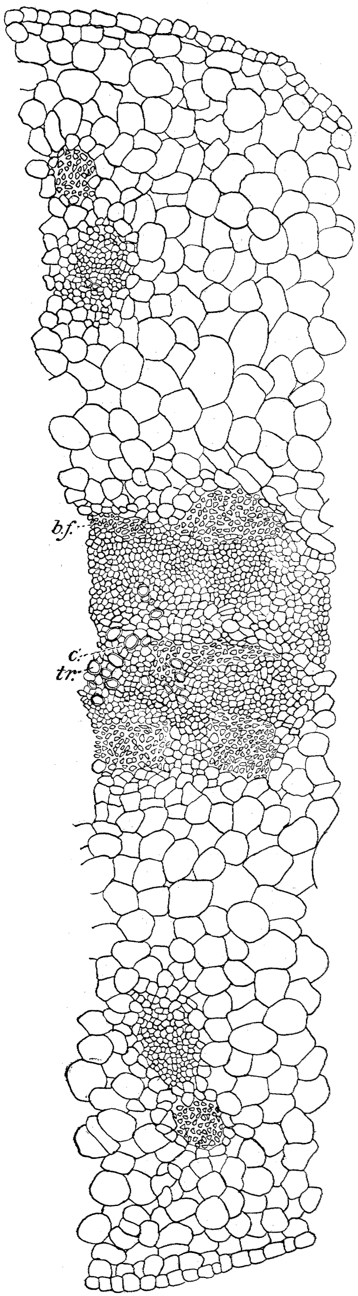

FIG. 1.

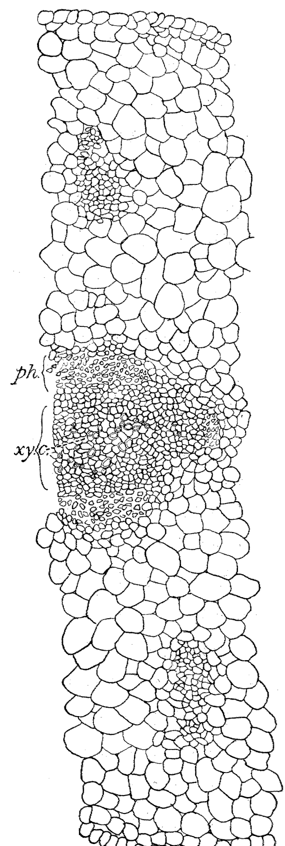

FIG. 2.

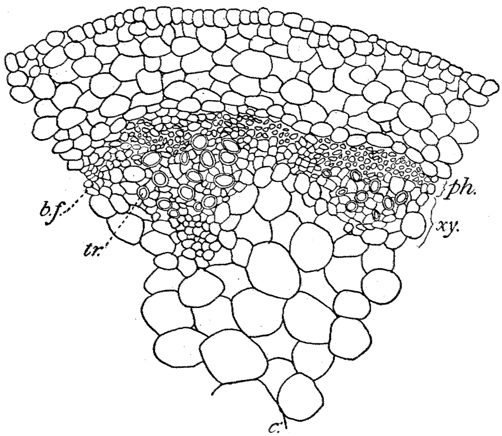

FIG. 3.

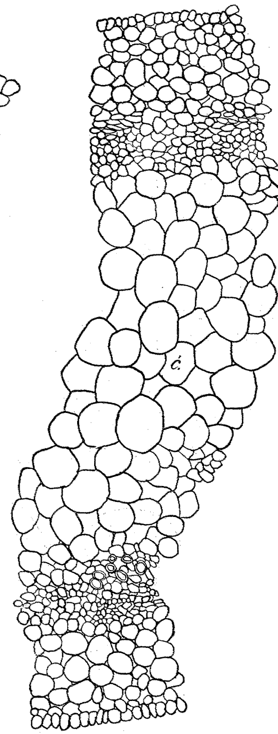

FIG. 4.

DESCRIPTION OF FIGURES.

b.f. = bast fibers. *tr.* = tracheid.
ph. = phloem. *xy.* = xylem.
c = center of stem.

FIG. 1. — Part of a cross-section of the stem of a normal pea seedling.
FIG. 2. — Part of a cross-section of the stem of a dwarf pea seedling.
FIG. 3. — Cross-section of a sector of the stem of a normal nasturtium seedling.
FIG. 4. — Part of a cross-section of the stem of a dwarf nasturtium seedling.

have been given and discussed were all drawn from three plants of each species, nevertheless that the general results are trustworthy is shown by another set of observations made on different pea plants. These gave the following results: Ratio of diameters of dwarf and normal plants, 111:62 (.56); cells in a given sector (7°), 165 in normal and 106 in dwarf (.64); cells in strips from center to circumference proportional in width to the size of the cross-section, 219 and 144 respectively (.66). The number of cells in a definite area was 29 in the normal and 44 in the dwarf. These measurements confirm those already discussed, for they show that the normal stem is larger in cross-section, is composed of a greater number of cells, and of larger cells than the dwarf.

The next question to be examined is the degree of differentiation of the various plants. This differentiation may be studied in the fibro-vascular bundles, where we may note the

appearance of the bundle as a whole, and the development of its elements. The appearance as a whole is indicated diagrammatically by the text-figures, which show especially marked differences in the case of the nasturtium. In the dwarf nasturtium the fibro-vascular elements are arranged in separate bundles around a central pith, while in the normal plant the phloem of the different bundles has run together, making a ring around the pith, the xylem being still discontinuous. Bast fibers seem highly developed with thick walls, and the tracheids are large, numerous, and clearly differentiated in the normal nasturtium stem ; while in the dwarf stem the soft bast has just begun to show signs of thickening into fibers, and the tracheids are small, and comparatively few and poorly differentiated. In both specimens of pea the fibro-vascular

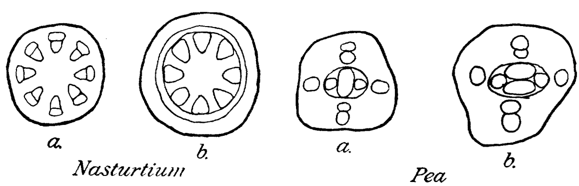

FIG. 5.

elements are arranged in an aggregate in the center of the stem and in four small groups peripheral to the large group. These bundles are more distinct and woody-looking to the naked eye in the normal than in the dwarf plants, and both bast fibers and tracheids are more numerous and highly differentiated. The normal plant, therefore, is more highly differentiated than the dwarf, as well as larger.

The conclusions reached from macroscopic and from microscopic examinations are then in accord with one another, and may be summarized by the statement that the removing of part of the cotyledons of a seed retards not merely the growth in size of the plant produced from that seed, but also its development. The plant, however, is not the counterpart of a younger normal plant, for it was found from comparing dwarf plants with check plants that the dwarf plant of a certain height was further developed than the check of the same height. The same point is illustrated by the fact that

a full-grown dwarf plant is smaller than a full-grown normal plant, as is shown by the nine-weeks stage of Table II.

The effect, it would seem, of removing a part of its food supply from the seed is not merely a transient one, but is one that can be traced through the whole life of the plant, and even increases as the plant grows older. The amount of food supply in the cotyledons influences, perhaps, the early stages of growth, while as the plant increases in size it becomes more and more vigorous and tends to grow more and more rapidly; so that a plant that is given an advantage over its fellow at the start will increase this advantage during subsequent development.